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Overcoming the challenges of applying target enrichment for translational research

by Andrew Barry, M.S., New England Biolabs, Inc.

Target enrichment is used to describe a variety of strategies to selectively isolate specific genomic regions of interest for sequencing analysis. The wide array of approaches presents challenges in selecting the appropriate technology for the growing number of research and clinical applications to which the sequencing data will ultimately be applied.

INTRODUCTION

In recent years, several techniques have emerged to enrich for specific genes of interest. When determining the appropriate target enrichment technology to use, one must first consider the primary goal of the study. For example, different approaches will be used if the aim is to identify known variants already shown to have clinical implications, versus discovering novel nucleic acid variants that may be associated with a given phenotype. Variant identification lends itself to more focused enrichment strategies, while variant discovery is driven by trade-offs between sequencing costs and target territory, as well as available sample cohort sizes for a given study.

As translational research seeks to bridge fundamental laboratory research and clinical treatment regimens for patients, there is an emerging need to balance discovery of novel nucleic acid variants, identification of known variants, and studies aimed at revealing associations with clinical phenotypes. Recent advances in sequencing technologies have revolutionized the field of genomic research, making tractable the application of whole genome and whole exome sequencing for broad discovery of germline genomic variants. However, despite these advances, the oncology field is fraught with the complexity of detangling the underpinnings of tumorigenesis, progression, and resistance mechanisms driven by somatic variants present at extremely low abundance in mixtures of malignant and stromal cells. These complexities necessitate increases in the depth of sequencing coverage to confidently call somatic variants, making broader scale approaches infeasible from an economic and practical standpoint.

To overcome these challenges, focused gene panels are being applied to patient samples. The size of the panel is highly variable, trending toward decreased genomic content as assays progress from pure research and discovery applications to clinical diagnostic assays. Furthermore, clinical applications raise the question of incidental findings and how to report them, introducing challenges for diagnostic assays based on sequencing entire genomes. This trend demonstrates the practical need for continued use of target enrichment strategies across the gamut of translational research activities.

TARGET ENRICHMENT APPROACHES

There are a number of different target enrichment approaches that can be grouped into three generalized categories: in-solution hybridization, multiplex PCR, and "alternative approaches", which span a wide variety of techniques.

In-solution hybridization-based approaches, originally developed for whole exome sequencing, use biotinylated oligonucleotides to capture genomic regions of interest (1). Commercially-available kits use DNA or RNA baits ranging from 50-150 nucleotides. Researchers have adapted this technique for more focused panels, ranging down to tens of kilobases in target territory, with limited success in maintaining specificity for target regions.

Multiplex PCR-based enrichment is most often employed for highly focused panels targeting a smaller territory than in-solution hybridization, and is typically limited to 150-200 amplicons (2). Using a pool of primers, enrichment is accomplished through PCR amplification of the targeted regions, which is followed by adaptor ligation or a second round of PCR using tailed primers to include sequencing adaptors. Scaling this technology has presented a challenge in maintaining target coverage uniformity.

A number of alternative approaches have been developed in an attempt to bridge the gap between hybridization and PCR-based approaches. Examples of these hybrid approaches include multiplex extension ligation (3), molecular inversion probes (MIPS)/padlock probes (4), nested patch PCR (5), and selector probes (6). These technologies can be broadly characterized as having more complex workflows, requiring splitting of samples into separate reactions, and creating challenges in target coverage uniformity.

NEBNext Direct® for target enrichment

NEBNext Direct is a novel target enrichment method that addresses several drawbacks that exist in alternative enrichment technologies (Table 1). Enrichment is achieved through direct hybridization of biotinylated DNA baits to denatured, fragmented molecules, which are subsequently captured using magnetic streptavidin beads (Figure 1, page 3). Unlike alternative in-solution hybridization protocols, the NEBNext Direct protocol does not require library preparation prior to hybridization of oligonucleotide probes. This feature reduces the overall amount of amplification that is required throughout the protocol and enables single-stranded DNA to be captured along with denatured, double-stranded DNA.

Conversion of captured fragments to sequence-ready libraries is achieved by the ligation of a loop adaptor to the proximal 3´ end of the captured molecule. During these steps, the bait / target molecules remain bound to the magnetic streptavidin beads and are processed in a single reaction tube. This eliminates sample loss and improves overall conversion efficiency.

TABLE 1:
Enrichment Challenges and Advantages of NEBNext Direct

Challenge	NEBNext Direct Advantage
Specificity across panel sizes	Enzymatic removal of off-target sequence
Uniformity of coverage	Individual synthesis of baits & empirical balancing
Sensitivity to detect variants	Unique Molecule Indexes for PCR duplicate marking & consensus variant calling
Degraded or low quality samples	Short baits that extend across molecules, targeting both DNA strands

Following ligation of the 3′ adaptor, the bait is extended across the entirety of the captured molecule, resulting in double stranded DNA that is ready for ligation of the 5′ unique molecular identifier (UMI) adaptor. This adaptor contains a 12 bp random sequence that is incorporated discretely into each molecule, indexing each molecule prior to amplification. This index can be used to identify duplicate molecules, thereby reducing artifacts that can lead to false positive variant calls.

Once the 5′ adaptor is ligated, the 3′ loop adaptor is cleaved, and the target molecule is PCR amplified off of the bait complex. It is important to note that the bait strand is not perpetuated through the PCR amplification and is not present in the final, sequencer-ready library.

The coverage plots of NEBNext Direct libraries are unique for a hybridization-based approach in that reads have a defined 3′ end, resulting in coverage plots that resemble PCR-based libraries, yet the approach allows for flexibility in tiling across longer targets. Disambiguation of PCR duplicates is accomplished by two features of the NEBNext Direct library: A variable 5′ end and a 12 bp randomized UMI that is incorporated into the 5′ adaptor.

CHALLENGES OF TARGET ENRICHMENT FOR TRANSLATIONAL RESEARCH

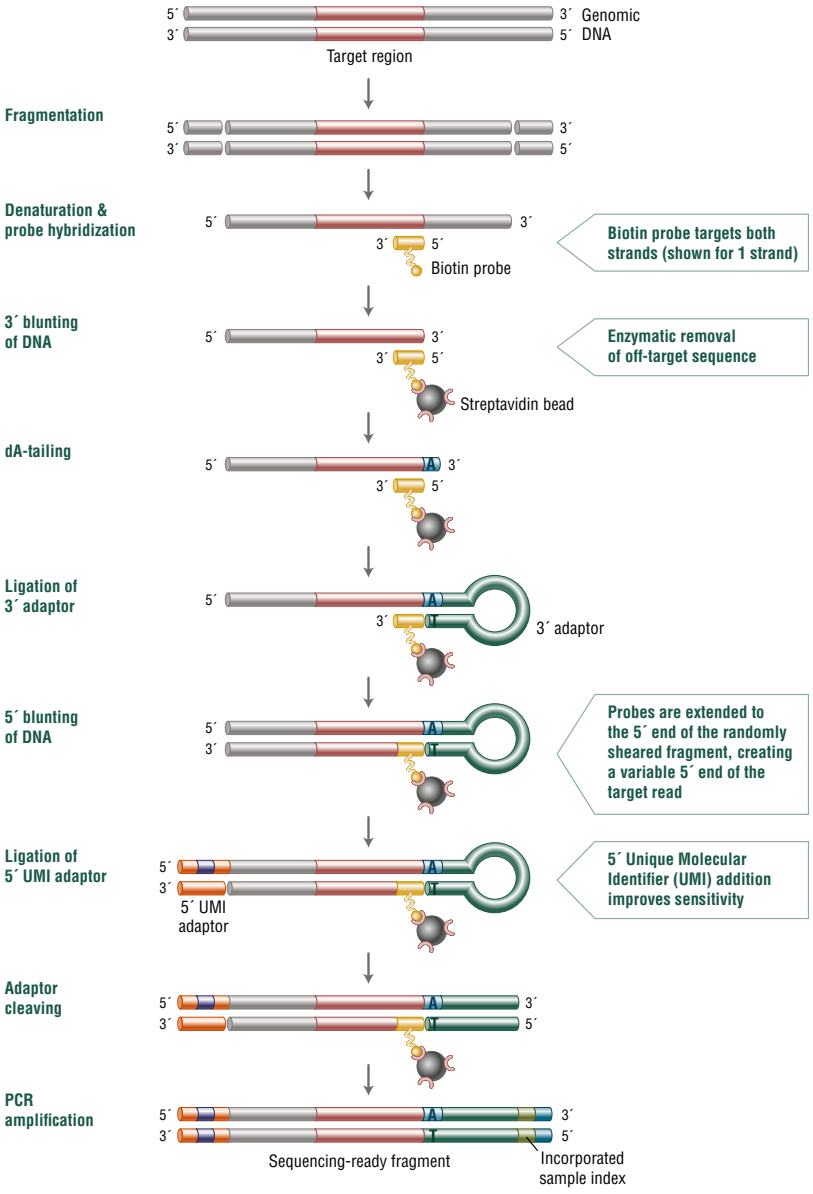
Specificity of target enrichment

For any study that necessitates enrichment of specific targets over more comprehensive sequencing approaches, specificity becomes more important as it directly translates to the amount of sequencing required to achieve the minimum coverage threshold to reliably detect variants of a given frequency. Specificity is typically measured by looking at the percentage of sequencing data that is derived from the targeted regions relative to the data that is aligned to other parts of the reference genome.

Enrichment of genomic regions is typically achieved by either amplifying the desired regions through PCR to generate enough copies of the targeted regions over the untargeted regions, or through hybridization of complementary biotinylated oligonucleotide probes to fragmented DNA molecules, where specificity is driven through careful control of melting temperatures and buffer composition to promote hybridization.

Specificity for target regions is enhanced using NEBNext Direct through both the hybridization of specific baits, as well as through enzymatic removal of off-target sequence. The enzymatic treatment removes both off-target sequence of molecules unbound to baits, as well as the regions of molecules upstream of where the baits are bound. This additional means of driving specificity enables the bait hybridization to be shorter, lasting only 90 minutes in duration. This differs from a typical hybridization-based approach,

FIGURE 1:
NEBNext Direct target enrichment workflow



in which randomly fragmented molecules are captured overnight, and without any removal of upstream off-target sequences, read coverage resembles a normal distribution.

While specificity for targeted regions using traditional hybridization approaches is typically quite high for larger panels up to whole exome, specificity typically decreases as the size of the targeted region decreases. Thus, smaller panels typically result in an increased proportion of sequencing lost to off-target regions. In contrast, the NEBNext Direct approach maintains high specificity across a broad range of target territory, from single genes or exons to hundreds of kilobases, eliminating the need to use different technologies for different panels (Table 2, page 4).

Uniformity of coverage across targets

One of the drawbacks to many available target enrichment methods is the inability to enrich different targets with equivalent efficiency. The result requires an increase in the overall coverage for all targets to achieve the minimum depth of coverage required to reliably call variants. One of the main factors influencing coverage unevenness is the sequence composition of the targeted regions themselves, with different efficiencies for sequences comprised of GC or AT rich regions.

Depending on the approach, the target enrichment strategy being employed may be more or less susceptible to the need for balancing melting temperatures across any complementary oligonucleotide baits or PCR primers that are employed in the enrichment process. Challenges

continued on page 4...

in uniformity can also arise from any downstream PCR that is used to generate sufficient material for the sequencing process, as various DNA polymerases demonstrate biases toward targets that may include secondary structure.

Using multiplex PCR-based workflows, primer design is limiting as melting temperatures must match within each panel and primer-primer interactions and primer cross-talk must be considered. These constraints can lead to variations in coverage uniformity between targets. Partitioning individual amplification reactions into emulsion droplets can alleviate some of these constraints and improve target uniformity (12), but this approach requires investment in instrumentation as well as additional workflow steps.

Oligonucleotides utilized during NEBNext Direct enrichment are individually synthesized, which enables bait pools to be carefully optimized based on empirical testing. Individual baits are balanced, allowing fine tuning of target coverage. Additionally, the bait design algorithm optimizes new bait design based on outcomes from prior results. Further, because the specificity is not solely driven through melting temperatures alone, NEBNext Direct allows increased flexibility in bait design.

The result is coverage across targets that can be optimized, demonstrating high degrees of uniformity and diminishing the overall amount of sequencing required to identify nucleic acid variants (Figure 2).

Sensitivity to detect nucleic acid variants

Perhaps the most critical aspect is the sensitivity of an approach to detect nucleic acid variants, as this is often the primary goal of studies in humans where target enrichment is employed. This is measured as the ability of an assay to detect nucleic acid variants that are present at a given frequency, referred to as variant allele frequency (VAF) or mutation allele frequency (MAF). Biologically, in the context of solid tumors, this is a function of the mixture of stromal and tumor cells, as well as the heterogeneity of tumor cells, and the existence of subclonal variants that are associated with tumorigenesis. Utilization of sequence data for the approximation of allele frequency is achieved through counting of sequence reads that possess a given variant. Quantitative assessment of sequence reads is challenged through the presence of duplicate molecules, or molecules that are identified through sequencing as having the same genomic coordinates. Depending on the target enrichment method that was employed to prepare the samples for sequencing, disambiguation of molecules that have arisen from discrete copies of genomic DNA versus those resulting from PCR amplification can be difficult or impossible to ascertain.

Disambiguation of PCR duplicates is accomplished by two features of the NEBNext Direct library: A variable 5´ end and a 12 bp randomized UMI that is incorporated into the 5´ adaptor. The amount of coverage one can expect from a given panel should be measured once duplicate molecules are removed in order to determine if the coverage is deep enough to reliably call a variant as a true-positive variant (Figure 3, page 5).

Difficult sample types

Whether for research or clinical applications, translational genomics often examines samples that are derived from patients. Patient tissue can be compromised by processes used to collect, preserve, store, extract nucleic acids from, and ultimately prepare for sequencing-based assays.

FIGURE 2:
NEBNext direct delivers higher coverage uniformity than alternative approaches.

Plot shows the uniformity across targets for each panel, measured as the percentage of bases above 25% of the mean target coverage. Samples were processed in duplicate according to the manufacturer's suggested protocol using the recommended amount of DNA input. DNA used was a blend of 24 HapMap samples. Samples were sequenced on an Illumina® MiSeq® per the manufacturer recommendation. Representative data across 2 replicates are shown.

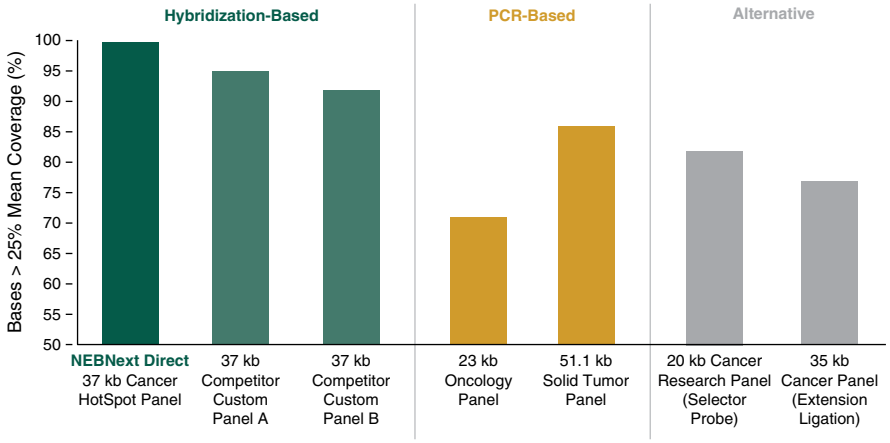


TABLE 2:
Specificity and uniformity of NEBNext Direct panels

Panel Size (kb)	Specificity (% Reads on Target)	Uniformity (% bp >20% MTC*)
15.2	99.4	99.3
15.9	96.1	100
20.4	99	99.5
36.8	92.5	98.7
76.4	91	98.5
93	95.9	99.35
217	90	99.23

** bp – base pairs MTC – Mean Target Coverage*

The most widely used technique for the storage and preservation of tissue derived from patient samples involves fixing the tissue in formalin, and embedding the fixed sample in paraffin. DNA derived from formalin-fixed, paraffin embedded (FFPE) samples has been shown to contain varying degrees of degradation, accumulation of base-specific errors, DNA breaks with damaged ends, and are often present in extremely low quantities (7-9). The recent application of target enrichment to circulating cell-free DNA molecules offers a less invasive means of monitoring cancer progression. Cell-free DNA derived from solid tumors is biologically present in relatively short fragments of 150-160 bp, which can present challenges using traditional enrichment approaches as both cell-free and FFPE tissue-derived nucleic acids contain high amounts of ssDNA (10, 11).

Using in-solution hybridization based enrichment presents challenges, as an upfront library must be prepared prior to hybridization to long (>100 bp) baits, and can result in sample loss. Moreover, degradation of FFPE derived nucleic acids can create shorter library inserts not optimal for hybridization to longer baits. Finally, the initial library generation step requires dsDNA; thus, the approach disregards ssDNA that may be present in the original sample due to DNA damage.

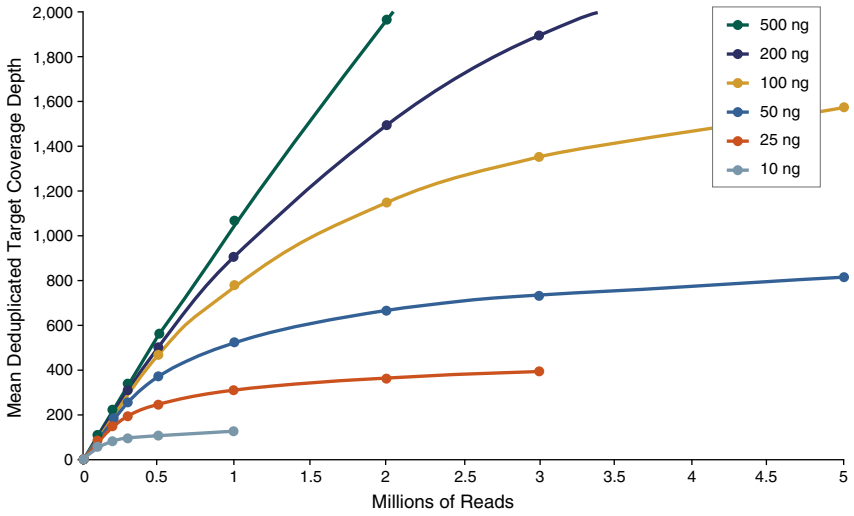
Multiplex PCR also presents challenges in targeting degraded samples, as the ability to successfully anneal both primers on a given molecule is difficult as DNA input molecule length is decreased due to degradation.

The short (~45-55 nucleotide) baits used in NEBNext Direct enrichment provide an increased probability of binding to shorter fragments, and the independent targeting of both strands of DNA offers improved opportunity to capture degraded fragments. The approach also contains an optional phosphorylation step to ensure the ends of target DNA are prepared for ligation of adaptors.

FIGURE 3:

NEBNext Direct is able to achieve high depths of sequence coverage across a broad range of inputs.

Mean depth of coverage relative to sequencing depth is shown across a range of DNA inputs. A blend of 24 HapMap samples were enriched using the 37 kb NEBNext Direct Cancer HotSpot Panel and sequenced on an Illumina MiSeq using 2 x 75 base pair sequencing. Coverage is shown after the removal of PCR duplicates using the information from the unique molecular identifier (UMI).



References

1. Gnirke, A., et al. (2009) *Nature Biotechnology* 27, 182–189.
2. Mertes, F., et al. (2011) *Briefings in Functional Genomics* 10, 374–386.
3. Shen, M.-J. R., et al. (2011) *Multiplex nucleic acid reactions.* US Patent 7955794 B2.
4. Porreca, G. J., et al. (2007) *Nature Methods* 4, 931–936.
5. Varley, K. E., and Mitra, R. D. (2008) *Genome Research* 18, 1844–1850.
6. Johansson, H., et al. (2011) *Nucleic Acids Research* 39:e8.
7. Srinivasan, M., et al. (2002) *The American Journal of Pathology* 161, 1961–1971.
8. Costello, M, et al. (2013) *Nucleic Acids Research* 41(6):e67.
9. Chen, L., et al. (2017) *Science* 355, 752-756.
10. Stiller, M., et al. (2016) *Oncotarget* 37(7), 59115-59128.
11. Burnham, P., et al. (2016) *Scientific Reports* 6, 27859.
12. Tewhey, R., et al. (2009) *Nature Biotechnology* 27, 1025–1031.

CONCLUSION

NEBNext Direct target enrichment overcomes several challenges translational researchers face in selectively enriching for certain genomic targets for clinical research. Providing the flexibility to use a single approach across a wide range of target content, NEBNext Direct allows enrichment of a single gene, up to panels comprised of hundreds of genes, without compromising performance as targets change. NEBNext Direct provides the specificity and coverage uniformity to maximize sequencing efficiency, in order to realize the benefits of target enrichment. Furthermore, intrinsic properties of the approach lend themselves to improved sensitivity, and have proven amenable to challenging sample types, typical of translational workflows. Combining the best aspects of hybridization-based enrichment and multiplex PCR enrichment, without the trade-offs, NEBNext Direct is a single-day, easy-to-use protocol that can be applied to advance translational research.

Learn more about NEBNext Direct at **NEBNextDirect.com**

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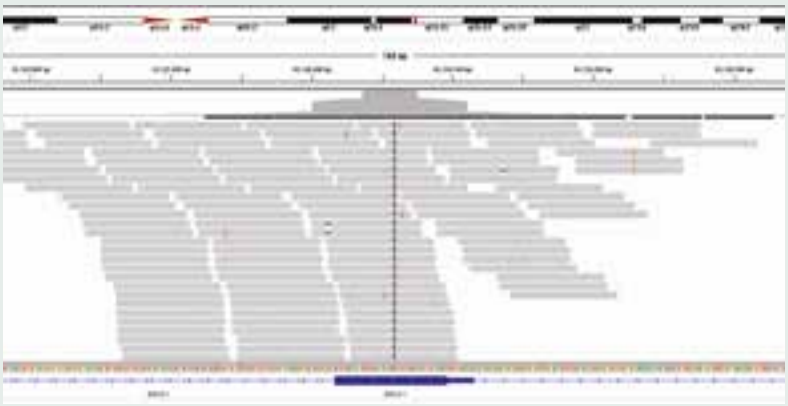
IGV plot showing complete and even coverage across exon 1 of the BRCA1 gene

Integrative Genomics Viewer (IGV) Plot of exon 1 from BRCA1 gene showing read level coverage obtained using 50 ng DNA enriched using the NEBNext Direct BRCA1/BRCA2 Panel. Sequencing was performed on an Illumina MiSeq using 2 x 75 paired-end sequencing.



Benefits

- Generate full (100%) coverage of all protein coding regions in BRCA1 and BRCA2 genes
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- Distinguish molecular duplicates, reducing false-positive variants and improving assay sensitivity
- Produce high depths of target coverage across a wide range of DNA input amounts for germline and somatic variants

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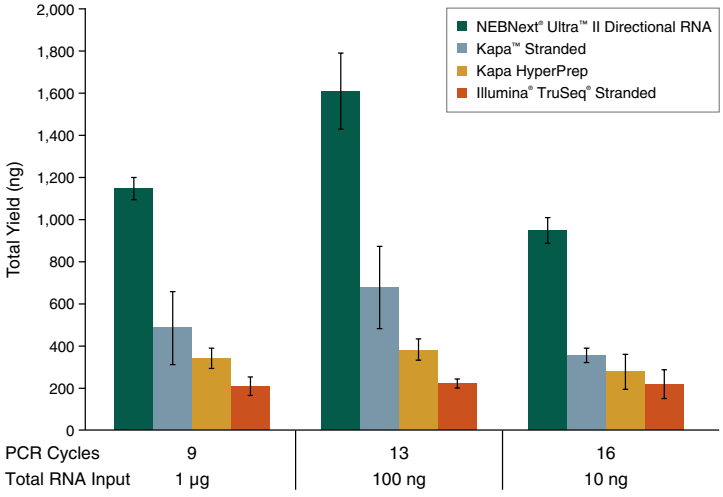

"The Ultra II RNA kit has allowed us to reduce the input for directional poly(A)+ RNA-seq libraries by a factor of 10 or more. We can now make a library with only 10 ng high quality total RNA and get the same gene expression profile as for 1 µg input. We've even pushed the input as low as 1 ng for very high quality total RNA. The new Ultra II RNA kit makes RNA-seq achievable for low yield samples, we actually need more RNA for quality control than for library prep! Furthermore, the library prep protocol is streamlined compared to the previous Ultra RNA kits, including a reduction in AMPure bead clean-ups and PCR cycles, resulting in better libraries for less time and resources."

– **Jen Grenier, Ph.D.**, Director of RNA Sequencing Core (RSC), Center for Reproductive Genomics, Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University

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1. Parkhomchuck, D., et al. (2009) *Nucleic Acids Res.* 37. e123.
2. Levin, J. Z., et al. (2010) *Nature Methods* 7, 709–715.

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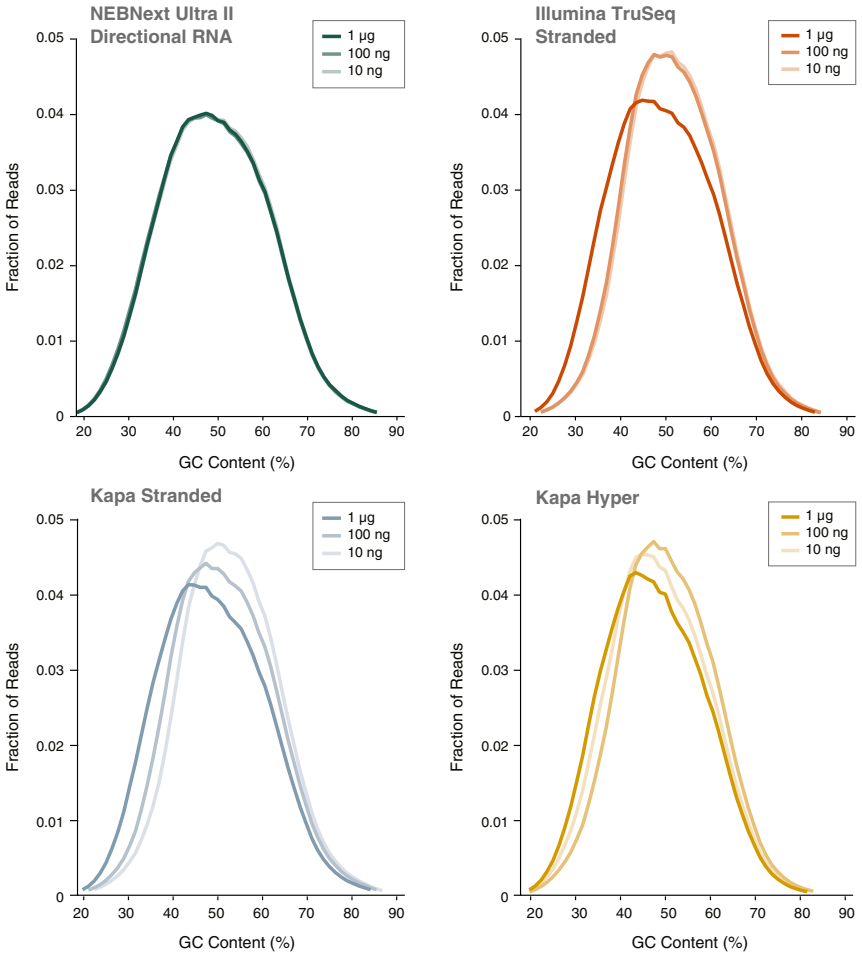
Poly(A)-containing mRNA was isolated from Universal Human Reference RNA (Agilent #740000), and libraries were made using the NEBNext Ultra II Directional RNA Kit (plus the NEBNext Poly(A) mRNA Magnetic Isolation Module), Illumina TruSeq Stranded mRNA Kit, Kapa Stranded mRNA-Seq Kit and Kapa mRNA HyperPrep Kit. Libraries were sequenced on an Illumina NextSeq® 500 using paired-end mode (2x76 bp). Reads were mapped to the hg19 reference genome. GC content distribution for each library was calculated using mapped reads. Ultra II Directional RNA libraries had uniform GC content distribution across a range of input amounts, as indicated by the curve overlap. For other kits the GC content distribution changed with different input amounts, indicating the introduction of input-dependent sequence bias.



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  NEB # E7765S/L **24/96 rxns**
- **NEBNext Ultra II RNA Library Prep Kit for Illumina**
  Includes optimized mixes for non-directional RNA library preparation (fragmentation, cDNA synthesis, end repair/dA-tailing, adaptor ligation and PCR enrichment steps) for sequencing on the Illumina platform.
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PRODUCT	NEB #	SIZE	PRICE
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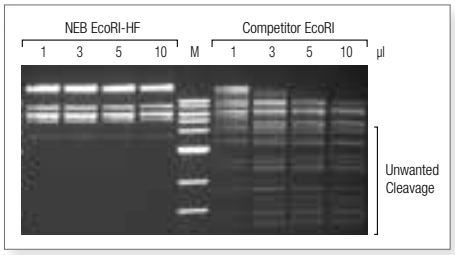

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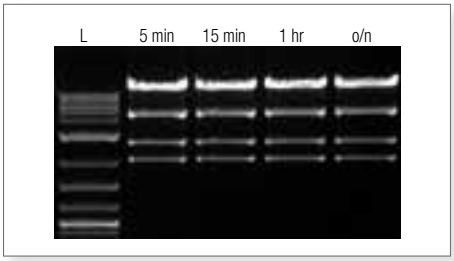
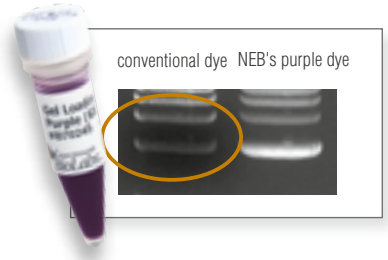
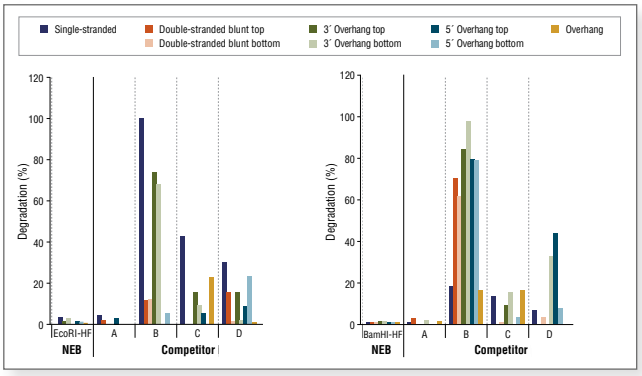


Optional Time-Saver digest:
pXba DNA was digested with EcoRV-HF according to the recommended protocol. Lane L is the 2-Log DNA Ladder (NEB #N3200). Complete digestion, free of unwanted star activity, is seen whether incubated for 5–15 minutes, 1 hour or overnight. For illustration purposes, the part of the gel below approx. 0.9 kb is not shown.



No UV-shadow and sharp bands:
Lane 1: UV shadow typical for conventional loading dyes (e.g. Bromophenol-Blue); Lane 2: NEB's purple loading buffer as supplied with all top selling restriction enzymes

NEB Restriction Enzyme Quality outperforms any competitor. Example: Nuclease Contamination.



EcoRI and BamHI from multiple suppliers were tested in reactions containing a fluorescent labeled single stranded, double stranded blunt, 3' overhang or 5' overhang containing oligonucleotides. The percent degradation is determined by capillary electrophoresis and peak analysis. The resolution is at the single nucleotide level.

see all details in new catalog:
p. 16 - 71 & appendix

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www.neb-online.fr

Advantages

- 285 restriction enzymes
- Unique engineered High-Fidelity (HF) restriction enzymes for superior performance
- Over 210 restriction enzymes are 100% active in a single buffer – CutSmart Buffer
- 194 restriction enzymes are Time-Saver qualified (5-15 min digest)
- All Top 35 modifying/cloning enzymes are 100% active in CutSmart Buffer
- Purple loading dye is included (product dependent)

Activity of DNA Modifying Enzymes in CutSmart Buffer: Clone smarter!

Enzyme	Activity in CutSmart	Required Supplements
Phosphatases:		
Alkaline Phosphatase (CIP)	+ + +	
Antarctic Phosphatase	+ + +	Requires Zn^{2+}
Quick CIP	+ + +	
Shrimp Alkaline Phosphatase (rSAP)	+ + +	
Ligases:		
T4 DNA Ligase	+ + +	Requires ATP
E. coli DNA Ligase	+ + +	Requires NAD
T3 DNA Ligase	+ + +	Requires ATP + PEG
T7 DNA Ligase	+ + +	Requires ATP + PEG
Polymerases:		
T4 DNA Polymerase	+ + +	
DNA Polymerase I, Large (Klenow) Frag.	+ + +	
DNA Polymerase I	+ + +	
DNA Polymerase Klenow Exo⁻	+ + +	
Bst DNA Polymerase	+ + +	
phi29 DNA Polymerase	+ + +	
T7 DNA Polymerase (unmodified)	+ + +	
Transferases/Kinases:		
T4 Polynucleotide Kinase	+ + +	Requires ATP + DTT
T4 PNK (3´ phosphatase minus)	+ + +	Requires ATP + DTT
CpG Methyltransferase (M. SssI)	+ + +	
GpC Methyltransferase (M. CviPI)	+	Requires DTT
T4 Phage β-glucosyltransferase	+ + +	
Nucleases, other:		
DNase I (RNase free)	+ + +	Requires Ca^{2+}
Endonuclease III (Nth), recombinant	+ + +	
Endonuclease VIII	+ + +	
Exonuclease III	+ + +	
Lambda Exonuclease	+ +	
McrBC	+ + +	
Micrococcal Nuclease	+ +	Requires Ca^{2+}
$RecJ_f$	+ + +	
T5 Exonuclease	+ + +	
T7 Exonuclease	+ + +	
USER™ Enzyme, recombinant	+ + +	

+ + + full functional activity + + 50–100% functional activity + 0–50% functional activity

A selection of DNA modifying enzymes were assayed in CutSmart Buffer and functional activity was compared to the activity in their supplied buffers. Reactions were set up according to the recommended reaction conditions, with CutSmart Buffer (plus required supplement) replacing the supplied buffer.

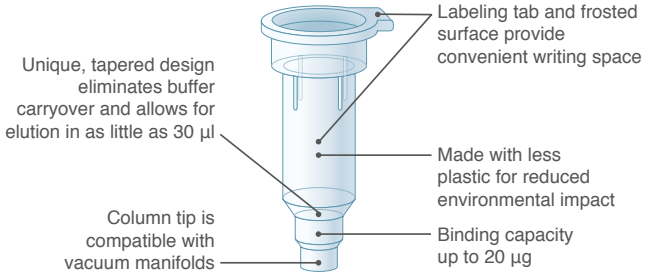
Tech Tip: When supplements are required, one can simply add the supplied buffer of the respective modifying enzyme at 1× concentration to the CutSmart Buffer to achieve appropriate activity for most applications – no change of buffers needed.

NEB: Zéro Sorcellerie

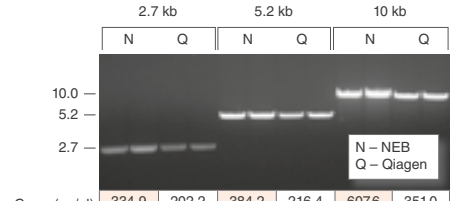
Designed for sustainability & performace:

Monarch Nucleic Acid Purification Kits from NEB

It's time to transform your DNA purification experience. NEB's Monarch Nucleic Acid Purification Kits are optimized for maximum performance and minimal environmental impact. Our unique thin-walled column design uses less plastic, prevents buffer retention, eliminates the risk of carryover contamination, and enables elution in smaller volumes. The result: high performing DNA purification for your downstream applications.

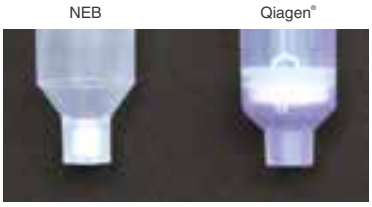


Monarch Plasmid Miniprep Kits consistently produce more concentrated plasmid DNA with equivalent yield, purity and functionality as compared to the leading supplier.



	2.7 kb		5.2 kb		10 kb	
	N	Q	N	Q	N	Q
Conc. (ng/µl)	334.9	202.2	384.2	216.4	607.6	351.0
Total yield (µg)	10.0	10.1	11.5	10.8	18.2	17.6
$A_{260/280}$	1.9	1.9	1.9	1.9	1.9	1.9
$A_{260/230}$	2.4	2.3	2.4	2.4	2.3	2.4

Designed for performance



Advantages

- Improved recovery of concentrated, pure DNA
- Low volume elutions, resulting in highly-concentrated DNA
- Fast, user-friendly protocols
- Significantly less plastic in every kit
- Responsibly sourced and recyclable packaging
- Reusable kit boxes
- Buffers and columns available separately

Free Sample

Request a free sample!**
Visit **NEBMonarch.fr**

25% discount* PROMO CODE: CLONING17

ORDERING INFORMATION:

PRODUCT	NEB #	SIZE	SPECIAL PRICE
Monarch DNA Gel Extraction Kit	T1020S/L	50/250 preps	66 € / 298 €
Monarch PCR & DNA Cleanup Kit (5 µg)	T1030S/L	50/250 preps	66 € / 298 €
Monarch Plasmid Miniprep Kit	T1010S/L	50/250 preps	56 € / 243 €

see all details in new catalog:
p. 140-145, 367

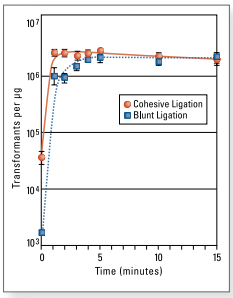
** Zéro Sorcellerie ! **Get 25% discount on selected NEB Cloning Products incl. all Restriction Enzymes** (Art.# Rnnnn)*
Eligible products are marked with the respective icon within this newsletter. See pages 8-13. Campaign ends Dec. 31st, 2017.
*Not cumulative with other discounts or special prices. Please enter the Promo Code "**CLONING17**" with your order.*

*** Limited supply. As long as stocks last.*

Clone with Confidence®!

Choose NEB's "Quick" DNA Ligases for performance & convenience

Fast & versatile: NEB's Quick Ligation Kit

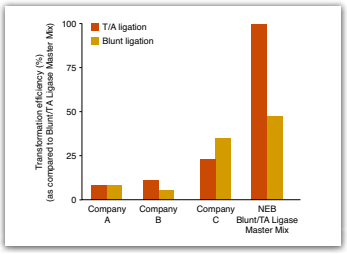


The Quick Ligation Kit enables ligation of cohesive end or blunt end DNA fragments in 5 minutes at room temperature (25°C)

Robust Ligation with No Incubation: Instant Sticky-end Ligase Master Mix

Sticky-ends ligations were set up using the Instant Sticky-end Ligase Master Mix. Without any additional incubation time, 2 µl were immediately withdrawn and used to transform a 50 µl aliquot of NEB 10-beta Competent E. coli (NEB #C3019).

Outperforms the competition: Blunt/TA Ligase Master Mix



Duplicate ligation reactions of blunt or T/A vector/insert pairs were set up according to the master mix vendors' suggestions. Equal amounts of ligated DNA were used to transform NEB 10-beta Competent E. coli (NEB #C3019).

Select the Ligase product that works best for you

DNA APPLICATIONS	QUICK LIGATION™ KIT	BLUNT/TA LIGASE MASTER MIX	INSTANT STICKY-END LIGASE MASTER MIX
LIGATION OF STICKY ENDS	★★★	★★	★★★
LIGATION OF BLUNT ENDS	★★★	★★★	★
T/A CLONING	★★	★★★	★

★★★ Optimal, recommended ligase for selected application
★★ Works well for selected application
★ Will perform selected application, but is not recommended

Advantages

- Industry standard for purity
- Highest reliability
- Fast ligations
- Robust ligation efficiency
- Convenient master mix formats

25% discount* PROMO CODE: CLONING17

ORDERING INFORMATION:

PRODUCT	NEB #	SIZE	SPECIAL PRICE
Quick Ligation Kit	M2200S/L	30 rxn (20 µl vol)/ 150 rxn (20 µl vol)	74 € / 297 €
Blunt/TA Ligase Master Mix	M0367S/L	50 rxn (10 µl vol)/ 250 rxn (10 µl vol)	74 € / 297 €
Instant Sticky-end Ligase Master Mix	M0370S/L	50 rxn (10 µl vol)/ 250 rxn (10 µl vol)	74 € / 297 €

see all details in new catalog: p. 107-113 & 362-366

TECH-TIP: Ligation of Vector and Insert

- Use a molar ratio of 1:3 vector to insert
- If using T4 DNA Ligase (NEB #M0202) or the Quick Ligation Kit (NEB #M2200), thaw and resuspend the Ligase Buffer at room temp. If using Ligase Master Mixes, no thawing is necessary.
- The Quick Ligation Kit (NEB #M2200) is optimized for ligation of both sticky and blunt ends
- Instant Sticky-end Ligase Master Mix (NEB #M0370) is optimized for instant ligation of sticky/cohesive ends
- Blunt/TA Ligase Master Mix (NEB #M0367) is optimized for ligation of blunt or single base overhangs, which are the more challenging type of ends for T4 DNA Ligase
- Following ligation, chill on ice and transform
- DO NOT heat inactivate when using the Quick Ligation Buffer or Ligase Master Mixes, as this will inhibit transformation
- Electroligase (NEB #M0369) is optimized for ligation of both sticky and blunt ends and is compatible with electroporation (i.e., no cleanup step required)

Ligation with the Quick Ligation Kit

Vector DNA (3 kb)	50 ng
Insert DNA (1 kb)	To 50 ng
2X Quick Ligation Buffer	10 µl
Quick T4 DNA Ligase	1 µl
Nuclease-free Water	20 µl (mix well)
Incubation	Room temperature for 5 minutes

Ligation with Instant Sticky-end Ligase Master Mix

Vector DNA (3 kb)	50 ng
Insert DNA (1 kb)	50 ng
Master Mix	5 µl
Nuclease Free Water	To 10 µl
Incubation	None

Ligation with Blunt/TA Ligase Master Mix

Vector DNA (3 kb)	50 ng
Insert DNA (1 kb)	50 ng
Master Mix	5 µl
Nuclease Free Water	To 10 µl
Incubation	Room temperature for 15 min

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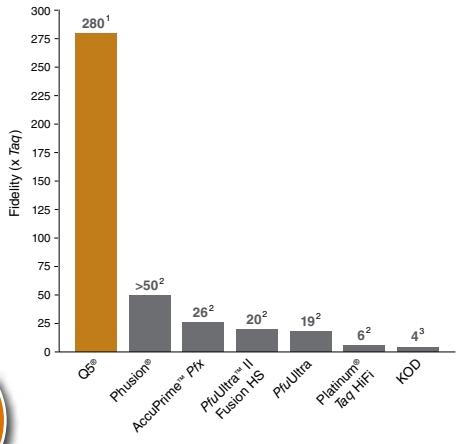
NEB: Clone with Confidence®!

Q5 High-Fidelity DNA Polymerase – Ultra fidelity for cloning & beyond

Q5 High-Fidelity DNA Polymerase is a high-fidelity, thermostable DNA polymerase with 3´-> 5´ exonuclease activity, fused to a processivity-enhancing Sso7d domain to support robust DNA amplification. With a fidelity >280-fold higher compared to Taq DNA Polymerase, Q5 High-Fidelity DNA Polymerase is ideal for cloning and can also be used for long or difficult amplicons. Q5 High-Fidelity DNA Polymerase is supplied with an optimized buffer system that allows robust amplification regardless of GC content.



Highest fidelity DNA amplification available. Fidelity of various PCR Polymerases as compared to *Taq* DNA Polymerase.

25% discount* PROMO CODE: CLONING17

ORDERING INFORMATION

PRODUCT	NEB #	SIZE	SPECIAL PRICE
Q5 High-Fidelity DNA Polymerase	M0491S/L	100/500 units	70 € / 312 €
Q5 Hot Start High-Fidelity DNA Polymerase	M0493S/L	100/500 units	82 € / 374 €

Advantages

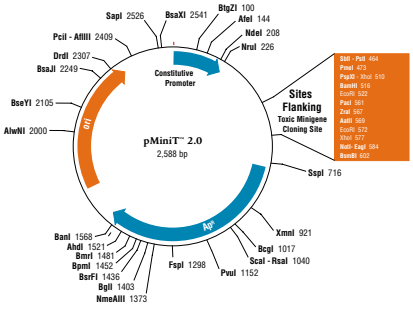
- **Fidelity** – the highest fidelity amplification available (> 280x higher than *Taq* and >4x higher than Phusion)
- **Robustness** – high specificity and yield with minimal optimization
- **Coverage** – superior performance for a broad range of amplicons (from high AT to high GC)
- **Speed** – short extension times
- **Amplicon length** – robust amplifications up to 20 kb for simple templates, and 10 kb for complex

see all details in new catalog: **p. 76-77**

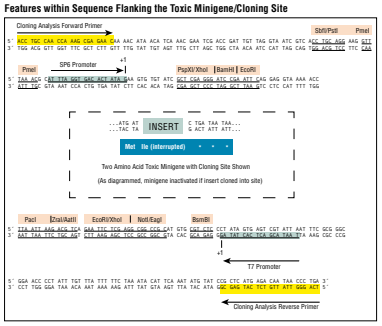
NEB PCR Cloning Kit: With or without competent cells

This PCR Cloning Kit (available with or without competent cells) contains an optimized Cloning Mix containing a proprietary ligation enhancer and a linearized vector that uses a novel mechanism for background colony suppression to give a low background. It allows simple and quick cloning of any PCR amplicon, whether the amplification reactions are performed with proofreading DNA polymerases, such as Q5 which produce blunt ends; or nonproofreading DNA polymerases, such as Taq which produce single base overhangs. This is possible due to "invisible" end polishing components in the master mix that are active during the ligation step only if needed. The kit also allows direct cloning from amplification reactions without purification, and works well whether or not the primers used in the PCR possess 5´-phosphate groups.

PCR cloning has never been easier!



Features within Sequence Flanking the Toxic Minigene/Cloning Site



Map shown above displays the construct formed if no insert is present. Unique restriction sites are shown in black. Additional restriction sites that can be used for subcloning are shown in red. Expanded box below shows location of sequencing primers, restriction sites for subcloning, and placement of insertion site within the toxic minigene.

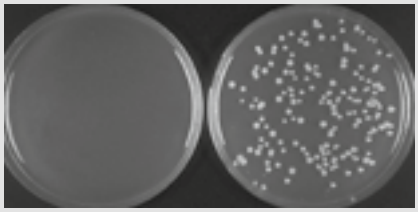
25% discount* PROMO CODE: CLONING17

ORDERING INFORMATION

PRODUCT	NEB #	SIZE	SPECIAL PRICE
NEB PCR Cloning Kit (without competent cells)	E1203S	20 rxns	105 €
NEB PCR Cloning Kit (incl. NEB10-beta competent cells)	E1202S	20 rxns	221 €

Advantages

- Works with both **blunt** and **TA ends**
- Clone faster, with **low/ no background**
- Get the colonies you need, with **high transformation efficiency**
- **No** need for **end-modification steps**
- **No blue/white screening** required
- New superior **Vector pMiniT 2.0** including SP6 and T7 promotors



PCR cloning with low/no background

A 500 bp PCR product was cloned using the NEB PCR Cloning kit (with competent *E. coli*). The left plate serves as the control, with vector backbone only. The right plate shows colonies containing the PCR insert.

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LUNA®

Universal qPCR & RT-qPCR Reagents

New England Biolabs is pleased to introduce a bright, new choice for your qPCR and RT-qPCR. Luna products have been optimized for robust performance on diverse sample sources and target types. Available for dye-based or probe-based detection, Luna products can be used across a wide variety of instrument platforms. With so many qPCR and RT-qPCR options available, why not make a simpler, more cost-effective choice that delivers the sensitivity and precision you expect for your qPCR and RT-qPCR.

A blue visible dye assists in tracking the reagents when pipetting into clear, multi-welled PCR plates.

EXPERIENCE BEST-IN-CLASS PERFORMANCE

- All Luna products have undergone rigorous testing to optimize specificity, sensitivity, accuracy and reproducibility.
- Products perform consistently across a wide variety of sample sources.
- A comprehensive evaluation of commercially-available qPCR and RT-qPCR reagents demonstrates superior performance of Luna products.

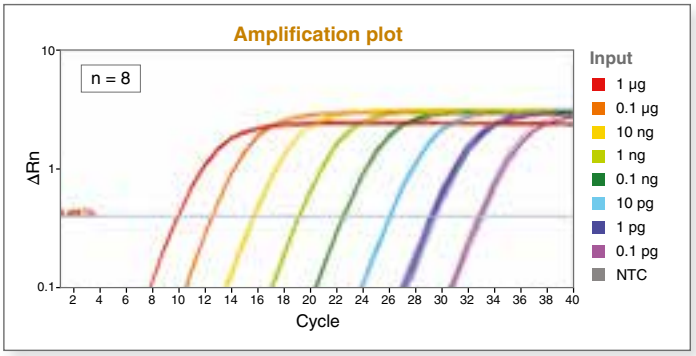
OPTIMIZE YOUR RT-qPCR WITH LUNA WARMSTART® REVERSE TRANSCRIPTASE

- Novel, thermostable reverse transcriptase (RT) improves performance.
- WarmStart RT paired with Hot Start *Taq* increases reaction specificity and robustness.

MAKE A SIMPLER CHOICE

- One product per application simplifies selection.
- Convenient master mix formats and user-friendly protocols simplify reaction setup.
- Non-interfering, visible tracking dye helps to eliminate pipetting errors.

Luna Universal One-Step RT-qPCR Kit offers exceptional sensitivity, reproducibility & RT-qPCR performance



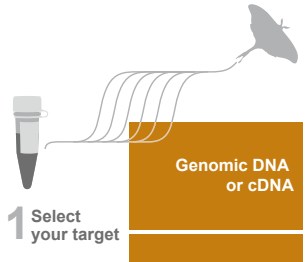
RT-qPCR targeting human GAPDH was performed using the Luna Universal One-Step RT-qPCR Kit (Input: 1 µg – 0.1 pg Jurkat total RNA); NTC = non-template control

see all details in new catalog:
p. 91-92 & 352-355

Free Sample

Request a free sample*!
Visit **LUNAqPCR.fr**

Find the right Luna product for your application:



1 Select your target	2 Select your detection method: Dye-based	Probe-based
Genomic DNA or cDNA	Luna Universal qPCR Master Mix (NEB #M3003)	Luna Universal Probe qPCR Master Mix (NEB #M3004)
RNA	Luna Universal One-Step RT-qPCR Kit (NEB #E3005)	Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006)

Ideally for high throughput users: Now also available in Extra-Large economic "E-Pack" sizes!

PRODUCT	NEB #	SIZE	PRICE
Luna Universal qPCR Master Mix	M3003S/L/X/E	200/500/ 1.000/2.500 rxns	113 € / 252 € / 450 € / 998 €
Luna Universal Probe qPCR Master Mix	M3004S/L/X/E	200/500/ 1.000/2.500 rxns	95 € / 212 € / 387 € / 860 €
Luna Universal One-Step RT-qPCR Kit	E3005S/L/X/E	200/500/ 1.000/2.500 rxns	234 € / 527 € / 915 € / 2.033 €
Luna Universal Probe One-Step RT-qPCR Kit	E3006S/L/X/E	200/500/ 1.000/2.500 rxns	211 € / 475 € / 833 € / 1.833 €

*Limit one per customer, while supplies last.

Choose NEB Competent Cells for your cloning

NEB's growing line of competent cells includes several popular strains for cloning and protein expression, in addition to strains with unique properties, including fast colony growth, tight control of expression and disulfide bond formation. Our cloning strains include derivatives of the industry standards, DH5α™ and DH10B™. NEB Turbo is unique to NEB, and produces visible colonies after only 6.5 hours of growth. NEB's dam⁻/dcm⁻ strain enables Dam and Dcm methylation-free plasmid growth. NEB Stable is recommended in most difficult cloning experiments. Our cells are all extensively tested for phage resistance, antibiotic resistance and sensitivity, blue/white screening and transformation efficiency. High efficiency, 5-minute transformation and electroporation protocols are provided, when applicable.

	NEB 5-ALPHA COMPETENT *E. coli* (#C2987)	NEB TURBO COMPETENT *E. coli* (#C2984)	NEB 5-ALPHA F´ I^q COMPETENT *E. coli* (#C2992)	NEB 10-BETA COMPETENT *E. coli* (#C3019)	*dam*⁻/*dcm*⁻ COMPETENT *E. coli* (#C2925)	NEB STABLE COMPETENT *E. coli* (#C3040)
FEATURES						
Versatile	•					•
Fast growth (< 8 hours)		•				
Toxic gene cloning		•	•			•
Large plasmid/BAC cloning				•		
Dam/Dcm-free plasmid growth					•	
Retroviral/lentiviral vector cloning						•
RecA	•		•	•		•
FORMATS						
Chemically competent	•	•	•	•	•	•
Electrocompetent	•	•		•		
Subcloning	•					
96-well format*	•					
384-well format*	•					
12 x 8-tube strips*	•					

** Other strains are available upon request. For more information, contact custom@neb.com.*

Advantages

- High transformation efficiencies
- Compatible with NEBuilder® HiFi DNA Assembly and Gibson Assembly® reactions, as well as ligation reactions
- Strains also available for cloning toxic genes
- All strains are free of animal products and T1 phage resistant
- Media and control plasmid is included
- Choose from a variety of convenient formats
- Bulk formats and custom packaging are available

Special Offer

Through January 31st, receive a free **NEB Tube Opener** with every competent cell purchase.**

FIGURE 1:
Benefit from high transformation efficiencies

Transformation efficiencies were compared using manufacturers' recommended protocols. Values shown are the average of triplicate experiments.

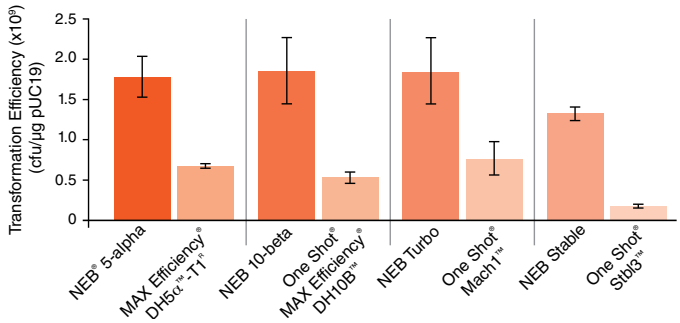
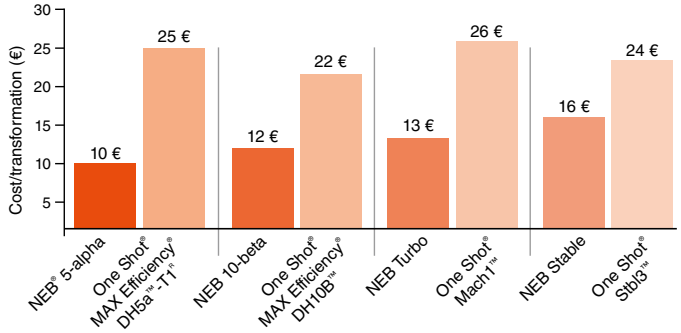


FIGURE 2:
Take advantage of the low cost per transformation

Calculations were based on published list prices as of 08/2017 and recommended transformation volumes. Prices may differ in various European countries.



**Limit one per customer, while supplies last. For more details, contact us at info.fr@neb.com

Enhancing Transformation Efficiency

Transformation efficiency is defined as the number of colony forming units (cfu) that would be produced by transforming 1 µg of plasmid into a given volume of competent cells. However, 1 µg of plasmid is rarely transformed. Instead, efficiency is routinely calculated by transforming 100 pg–1 ng of highly purified supercoiled plasmid under ideal conditions. Transformation Efficiency (TE) is calculated as: TE = Colonies/µg/Dilution. Efficiency calculations can be used to compare cells or ligations. Our recommended protocols and tips to help you achieve maximum results are presented here:

TRANSFORMATION TIPS

Thawing

- Cells are best thawed on ice
- DNA should be added as soon as the last trace of ice in the tube disappears
- Cells can be thawed by hand, but warming above 0°C decreases efficiency

Incubation of DNA with Cells on Ice

- Incubate on ice for 30 minutes. Expect a 2-fold loss in TE for every 10 minutes this step is shortened.

Heat Shock

- Both temperature and time are specific to the transformation volume and vessel. Typically, 30 seconds at 42°C is recommended.

Outgrowth

- Outgrowth at 37°C for 1 hour is best for cell recovery and for expression of antibiotic resistance. Expect a 2-fold loss in TE for every 15 minutes this step is shortened.
- Use NEB 10-beta/Stable Outgrowth Medium for 10-beta and Stable Competent *E. coli*. Use SOC for all other strains.
- Outgrowth medium gives 2-fold higher TE than LB medium
- Incubation with shaking or rotation results in 2-fold higher TE

Plating

- Selection plates can be used warm or cold, wet or dry with no significant effects on TE
- Warm, dry plates are easier to spread and allow for the most rapid colony formation

DNA

- DNA should be purified and resuspended in water or Tris-EDTA Buffer
- Up to 10 µl of DNA from a ligation mix can be used with only a 2-fold loss of efficiency
- Purification by either a spin column or phenol/chloroform extraction and ethanol precipitation is ideal
- The optimal amount of DNA is lower than commonly recognized. Using clean, supercoiled pUC19, the efficiency of transformation is highest in the 100 pg–1 ng range. However, the total colonies which can be obtained from a single transformation reaction increase up to about 100 ng.

DNA CONTAMINANTS TO AVOID

CONTAMINANT	REMOVAL METHOD
Detergents	Ethanol precipitate
Phenol	Extract with chloroform and ethanol precipitate
Ethanol or Isopropanol	Dry pellet before resuspending
PEG	Column purify or phenol/chloroform extract and ethanol precipitate
DNA binding proteins (e.g., ligase)	Column purify or phenol/ chloroform extract and ethanol precipitate

See all details in new catalog:
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Featured Videos

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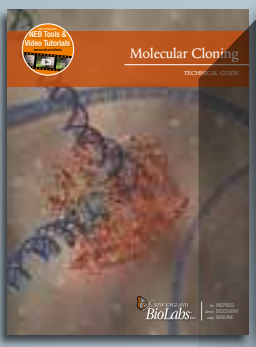

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